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Complement membrane attack (MAC) in idiopathic IgA-glomerulonephritis

ERNST W. RAUTERBERG, HANS–MICHAEL LIEBERKNECHT, ANNE–MARGRET WINGEN, and EBERHARD RITZ

Institute of Immunology and Department of Internal Medicine, University of Heidelberg, Federal Republic of Germany

Complement membrane attack (MAC) in idiopathic IgA-glomerulonephritis. Antigens of the membrane attack complex of complement (MAC), such as C5, C6, C9 and MAC-related neoantigen(s), were demonstrated in the mesangium of 23 cases with IgA-glomerulonephritis (IgA-GN) and two cases with Henoch–Schönlein purpura nephritis (HSP). High specificity of the polyclonal antibodies was verified by dot–blot analysis. Control specimens lacking immunoglobulin deposits were negative for MAC-related antigens. Markers of classical pathway activation (C1q and C4) were observed only in two of 24 and one of 23 cases of IgA-GN and HSP, respectively. Glomerular distribution patterns (mesangial vs. mesangio-peripheral) of immunoglobulin or complement deposits were correlated for IgA and C3b/iC3b ($P < 0.002$), for IgA and properdin ($P < 0.002$) and for IgA and MAC neoantigens ($P < 0.01$). Double immunostaining experiments revealed co-localization of IgA and MAC neoantigens at identical mesangial and capillary sites. Glomerular distribution of the less pronounced IgG or IgM deposits did not correlate with that of any complement–derived antigen. The pattern of MAC-related antigens was found to be uniformly either mesangial or mesangio-peripheral. Staining for MAC-related antigens was less intense in IgA-GN cases with minimal glomerular lesions than in cases with more advanced non-sclerosing lesions. IgA, C3d, and MAC localized in corresponding glomerular sites. This is consistent with complete local activation of complement by glomerular IgA deposits via the alternative pathway. The possibility exists that MAC plays a pathogenetic role, such as by irritation of bystander cells, in IGA-GN and HSP.

IgA mesangial glomerulonephritis (IgA-GN) is the most frequent form of glomerulonephritis. The characteristic immunopathological findings, mesangial or mesangio-peripheral granular deposits of IgA with or without concomitant IgG and IgM deposits, were first described by Berger and Hinglais in 1968 [1] and later confirmed by many investigators [2–4]. Similar histopathological features are observed in Henoch–Schönlein purpura nephritis (HSP) [5]. Although much effort has been invested to clarify the pathomechanisms involved, little information with respect to pathogenesis is available. It has not been clarified whether deposits derive from soluble immune complexes (IC), consisting of IgA and unknown antigens, such as from the gastrointestinal tract, or whether they represent IgA polymers or oligomers containing no antigen. IgA polymers or IgA-IC have been detected in the serum of patients with IgA-GN or HSP at least in the early stages of the disease [6–12]. However, the factors causing IgA-IC or IgA aggregates to be trapped within the mesangial area remain to be elucidated.

Finally, the events by which local deposition of IgA leads to recurrent hematuria, glomerular lesions and progressive renal failure are still unclear. One potential system mediating local tissue damage is the complement system. Its involvement in IgA-GN is suggested by the simultaneous occurence of granular C3b deposits with mesangial IgA (and IgG or IgM) deposits on immunofluorescence (IF) [2–4]. Such local mesangial complement activation may cause tissue damage by one of these possibilities (or their combination): local release of the potent anaphylatoxic factors C4a, C3a, C5a, which trigger acute inflammatory responses via cellular (polymorphonuclear leucocytes, thrombocytes or monocytes) and other mechanisms and of which C5a has potent chemotactic activity, and formation of the cytolytic membrane attack complex of complement (MAC), which could directly destroy or irritate bystander cells in the vicinity of the activation site or modify polyanion charge of glomerular basement membrane.

Since accumulation of PMNs or monocytes is not regularly detected in IgA-GN even during hematuric episodes, a pathogenic role for C-derived anaphylatoxins is less probable. This focused attention on the second possibility, the involvement of the membrane attack complex. Therefore, the present study was carried out to address the following questions: 1) Can MAC deposits be detected in glomeruli of patients with IgA-related glomerulonephritis (idiopathic IgA-GN or HSP)? 2) Are IgA and MAC localized in the same glomerular sites? 3) Is the pattern and the amount of MAC deposits, as assessed semiquantitatively by indirect immunofluorescence, correlated with histopathological features or with clinical findings (such as hematuria and/or proteinuria) at the time of biopsy?

Immunohistological identification of MAC was based on the demonstration of C5, C6, C9 by their respective antibodies and, in addition, by an antiserum specific for neoantigen(s) present on MAC but not on its single components C5, C6, C7, C8 and C9. MAC neoantigens are exposed upon the assembly of the late complement components with the formation of MAC [13]. Such antiserum against MAC-neoantigens is an elegant probe to demonstrate complement activation involving the terminal steps of the cascade.

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The results of this study indicate complete in situ activation of the complement system in IgA-related glomerulonephritis.

Methods

Patients and biopsy material

Kidney biopsy specimens sent to the Institute of Immunology, Heidelberg, between 1975 and 1980 and classified as IgA-GN or HSP were evaluated in this retrospective study. All specimens had been snap–frozen immediately after biopsy, embedded in Tissue Tec (Miles Laboratories, Naperville, Illinois, USA) in isopentane, cooled to its freezing point by liquid nitrogen, and stored at −85°C until used in the present study. The diagnosis of IgA-GN and HSP, respectively, was based on the demonstration of deposits of IgA (with or without IgG, IgM and C3) in the mesangial area of the glomeruli by immunofluorescence (IF) microscopy in the absence of liver disease or SLE. Clinical features were compatible with the diagnosis in all cases (Table 1). Biopsies without immunohistological findings on routine examination served as controls. These patients had been biopsied because of clinical signs suggesting glomerular disease, but had no glomerular deposits on immunofluorescence.

A total number of 29 cases were re-examined. The clinical features at presentation, selected clinical data at time of biopsy and the results of the histopathological evaluation are summarized in Table 1.

Antisera

Rabbit anti-human IgG/FITC, anti-human IgA/FITC, anti-human IgM/FITC, anti-human fibrinogen/FITC, anti-human C1q/FITC, anti-human C4/FITC, anti-human C3c/FITC, unlabeled anti-human C9 and anti-human S-protein were obtained from Behringwerke (Marburg, FRG). Rabbit anti-human fibronectin was purchased from Cappel Laboratories (Conchranville, Pennsylvania, USA). Unlabeled rabbit anti-human C5 and anti-human C3d/FITC were obtained from Dakopatts (Copenhagen, Denmark) and goat anti-human factor B/FITC, anti-human factor H/FITC, anti-human factor P/FITC from Atlantic Antibodies (Scarborough, New York, USA). Anti-rabbit C6 cross–reacting with human C6 was raised in C6 defective rabbits as described [14]. Anti-C3d/FITC was affinity purified using C3d bound to thiol sepharose according to Ross et al [15]. Using C3b and C3d coated sepharose beads, the titers of anti-human C3b and anti-human C3d as evaluated by serial dilution were similar after appropriate adjustment, and yielded comparable sensitivity of IF. Specificity of anti-human C3c was verified by showing no cross–reaction with C3d coated beads, whereas anti-human C3d gave a faint reaction with C3b and an intensive one with C3d-coated beads.

Specificity of anti-C5, anti-C6 and anti-C9 was verified by dot–blot analysis using purified components and by SDS-PAGE immunoblotting using purified components and plasma as antigen. All three antisera showed only minimal contaminating antibodies against those other than the stated antigen with the latter technique (which in our laboratory had a higher sensitivity than IF).

In indirect immunofluorescence (IF) tests with unlabeled primary antibodies, swine anti-rabbit IgG/FITC from Dakopatts or rabbit anti-goat IgG/FITC were used at a dilution of 1:10. Normal rabbit IgG/FITC or normal goat IgG/FITC showed no staining when used as controls for direct IF. Similarly, when the primary antibody was omitted, FITC labeled secondary antibodies failed to stain.

Antibodies against neoantigens present on the membrane attack complex of complement (anti-MAC-neo) were produced in rabbits as described previously [16].

Reactivity of the anti-MAC-neo was examined by several independent techniques.

First, intermediate gel crossed immunoelectrophoresis technique was applied. Using yeast or zymosan activated NHS as antigen, one precipitation arc appeared, which moved down into the intermediate gel when anti-C5 and anti-C9 were present; no precipitation occurred there with fresh human serum. This indicates that the complexes precipitated by anti-MAC carry also C5 and C9 epitopes.

Second, when immunoferritin staining with cells bearing various C5-9 intermediates was examined, such as EC5b67, EC5b678, and EC5b6789, our anti-MAC-neo mainly reacted with neoantigens present on the C5-8 part of MAC [17]. Complement lysed erythrocyte membranes incubated with anti-MAC-neo and ferritin–labeled secondary antibodies revealed (after negative staining) a high association of the ferritin label to the complement lesions [16].

Third, no reactivity of anti-MAC-neo with plasma proteins, yeast–activated serum proteins or purified MAC was found when using SDS-PAGE immunoblotting technique. We interpret this as evidence that anti-MAC-neo recognized epitopes requiring specific protein conformations which are lost on SDS-PAGE.

Fourth, selective specificity for MAC was proven using dot–blot analysis: from complement activated serum, SC5b-9 complexes were separated from uncomplexed components by gelfiltration on Superose™ 6 column (FPLC, Pharmacia Diagnostics, Uppsala, Sweden). As controls, fresh human sera (NHS) were subjected to gelfiltration under identical conditions. In the fractions collected, reactivity with anti-MAC, anti-C5, anti-C9, anti-IgM, anti-IgG and anti-albumin was measured by dot–blot analysis. Antigenic activities of C5 and C9 were found as one peak each in the late inclusion volume of NHS. Anti-MAC-neo did not react with fractions of NHS. With activated serum an additional antigenic activity of C5 and C9 appeared in the void volume (and fractions following thereafter in the early inclusion volume but distinct from the respective peaks of free C5 and C9). Such additional antigenic action of C5 and C9 coincided with anti-MAC-neo reactivity. We conclude that the antibodies are directed against neoantigens present only on the C5b-9 complex.

Fifth, anti-MAC-neo reacted with SC5b-9 isolated by ammonium sulfate precipitation, gel filtration, and FPLC mono-Q ion–exchange chromatography (purity proven by SDS–PAGE) in dot–blot analysis even in the presence of human plasma or polyclonal $F(ab)_2$-fragments of rabbit anti-S protein in excess.

Sixth, absorption of anti-MAC-neo with purified SC5b-9 complexes completely abrogated the reactivity with MAC-positive kidney biopsy specimen.

The IgG fraction of the anti-MAC-neo was prepared by ion exchange chromatography [18] and used for indirect staining.

Table 1. Clinical features of 23 patients with IgA-gomerulonephritis, 2 patients with Henoch–Schönlein purpura and 4 control patients

Patient no.	Age at onset/ sex	Age at biopsy	Presenting symptoms[a]	Course before biopsy[b]	At time of biopsy: Urinary protein[c]	Blood pressure *mm Hg*	Serum creatinine *mg%*	Histopathological feature[f]
IgA-glomerulonephritis								
1	5F	6	gross hematuria	IM	no	110/70	0.5	MGL
2	56M	56	microhematuria	IMX	no	140/80	3.0[e]	FSGN
3	37M	39	microhematuria, hypertension	IM	no	140/95	1.6[e]	MGL
4	19M	20	proteinuria	IM	>1g/day	180/100[d]	0.8	FSGN
5	12M	13	gross hematuria	IM	<1g/day*	120/70	0.9	FSGN
6	18M	20	microhematuria	IX	<1g/day	130/80	0.8	MGL
7	36M	37	gross hematuria, proteinuria	IMX	no	120/80	0.8	MGN
8	30M	31	hypertension	ND	<1g/day	160/110[d]	1.7[e]	GS
9	23M	29	proteinuria, microhematuria	PPM	<1g/day	130/70	1.1	MGN
10	9M	15	gross hematuria	IMX	no	125/80	0.7	FSGN
11	9M	11	edema, gross hematuria	PM	<1g/day	130/90	0.7	EEGN
12	11M	12	microhematuria, proteinuria	PM	no	125/80	0.7	MGL
13	33F	33	gross hematuria	IMX	no	110/80	0.9	MGL
14	22F	23	gross hematuria	IM	<1g/day	140/80	0.7	MGL
15	28M	29	microhematuria	IMX	<1g/day	110/70	1.2	MGN
16	45M	46	gross hematuria	IMX	>1g/day	210/130[d]	1.9[e]	MGN
17	9M	11	gross hematuria	IMX	no	120/90	0.7	FSGN
18	9M	12	gross hematuria	IPM	>1g/day	130/90	0.7	EEGN
19	40M	48	hypertension, gross hematuria	IMX	>1g/day	170/110[d]	1.0	MGN
20	23M	41	microhematuria	IM	<1g/day	135/90	1.3	MGN
21	15M	15	microhematuria	PM	<1g/day	140/70	0.8	MGL
22	18M	19	proteinuria, microhematuria	IMX	<1g/day	130/90	1.2	MGN
23	29F	33	proteinuria	PPM	>1g/day	130/85	1.4[e]	MGN
Henoch–Schönlein purpura nephritis								
24	9M	9	purpura	IPM	no	120/70	0.8	FSGN
25	8F	10	purpura	PMX	no	120/80	0.4	MGN
Controls: immunohistologically negative cases								
26	4M	5	proteinuria	PPM	<1g/day	140/90	0.7	MGL
27	37M	37	gross hematuria	ARF	<1g/day	170/120[d]	2.1[e]	MGN
28	31M	31	edema	ARF	<1g/day	140/90	5.9[e]	MGN
29	6F	10	gross hematuria	IM	<1g/day	110/60	0.7	MGL

[a] First symptoms: hypertension, blood pressure >140/90 mm Hg; gross hematuria, >10.000 RBC/μl; microhematuria, microscopic hematuria; proteinuria, >150 mg/day

[b] Abbreviations are: IM, intermittent microscopic hematuria; IX, intermittent macroscopic hematuria; IMX, intermittent microscopic and gross hematuria; IPM, intermittent proteinuria and microscopic hematuria; PM, persistent microscopic hematuria; PPM, persistent proteinuria and microscopic hematuria; PMX, persistent microscopic hematuria with macroscopic episodes; ND, no data available

[c] Proteinuria, *higher in the course before biopsy; no <150 mg protein/1.73 square meter/day; <1g/day 150 – 1000 mg protein/day (for children normalized/1.73 square meter/day); >1g/day more than 1g protein/day (for children normalized/1.73 square meter/day)

[d] Elevated, >140/90 mm Hg

[e] Above 1.3 mg% in adults or above range for sex and age matched controls given by Goldsmith [28]

[f] Abbreviations are: MGL, minimal glomerular lesions; FSGN, focal and segmental glomerulonephritis; MGN, mesangial proliferative glomerulonephritis; EEGN, endo- and extracapillary proliferative glomerulonephritis; GS, diffuse glomerulosclerosis

Immunofluorescence microscopy

Cryostat sections (4 μm) of specimens were fixed for 10 minutes in acetone, air dried, and incubated with the respective FITC-labeled antibodies diluted 1:20 in PBS (except anti-fibronectin 1:5) or with unlabeled antibodies diluted 1:10 (except: anti-MAC-neo undiluted). After incubation and washing steps according to the routine protocol for IF [19] the sections were mounted in PBS-glycerol. In case of unlabeled first antibodies the appropriate FITC-labeled second antibodies were used. Secondary antibodies failed to react with human tissue in control sections.

Specimen sections with at least one glomerulus (range 1 to 40, mean 7) were examined in a Zeiss Standard 18 fluorescence microscope equipped with a vertical epi-illuminator (lamp: HBO 50; excitation filter 485 + 10 nm; barrier filter 515 nm) (Zeiss, Oberkochen, FRG). The immunohistological (IF) findings in each specimen were assessed and scored with respect to the following criteria by two independent observers who were unaware of the diagnosis: (a) total number of glomeruli; (b) number of affected glomeruli; (c) localization of glomerular deposits, that is, deposits along the glomerular basement membrane (peripheral), purely mesangial (mesangial), mainly mesangial with additional spreading into the peripheral base-

ment membrane (mesangio-peripheral); (d) size of mean deposits (fine, intermediate or coarse granular); (e) overall fluorescence intensity of the deposits per glomerulus; that is, weak (1+), or marked (2+) or extensive (3+).

The estimated scores (1+, 2+, or 3+) were taken to calculate the sum of the scores for C5, C6, C9 and MAC neoantigens staining (Table 2). Photomicrographs from at least one representative glomerulus per antibody and per section were taken at a magnification of 125× and at constant exposure time (32 sec) using Kodak Ektachrom 400 ASA films (Eastman Kodak, Rochester, New York, USA).

In experiments to examine whether IgA and MAC are deposited in the same glomerular site, one glomerular section was simultaneously examined with anti-MAC-neo and IgA antibodies. First, sections were incubated with $F(ab)_2$-fragments/FITC of goat-anti-human IgA (Jackson Dianova, Hamburg, FRG) followed by rabbit-anti-MAC-neo and alkaline–phosphatase (AP)-labeled $F(ab)_2$ fragments of goat-anti-rabbit IgG. Omission of anti-MAC-neo gave negative AP staining results. Further experiments were done using in the second–step mouse monoclonal antibodies against the C9a fragment of C9 obtained by alpha–thrombin cleavage (Dr. Kroll, Bayer Co., Leverkusen) or monoclonal antibody against C5b neoantigen non-reactive with native C5 (20; unpublished material). For these two antibodies the APAAP technique [21] was used.

Statistics

In cases where all four stainings could be performed ($N = 16$), the sum of the scores for C5, C6, C9 and MAC neoantigens were used to correlate the amount of MAC with the histopathological features and clinical findings, that is, proteinuria (no/yes) or renal function (serum creatinine) by the rank sum test (Wilcoxon).

The relations between IF findings and histopathological lesions or clinical features were examined using chi–square tests. A conservative level of $P < 0.02$ was chosen as cut–off level for statistical significance.

Results

Immunoglobulin deposits in IgA-glomerulonephritis

The immunohistological findings are summarized in Table 2. By definition, in all biopsy specimens from patients with IgA-glomerulonephritis, IgA was the predominant immunoglobulin class diffusely deposited in the mesangial area of the glomeruli. The pattern of IgA deposition varied between purely mesangial (m, 14 of 23 cases) and mesangio-peripheral (mp, 9 of 23 cases); the latter is illustrated in Figure 1.

Additional IgG and/or IgM deposits of lower intensity were observed in all 23 patients (Table 2).

Deposits of complement factors in IgA glomerulonephritis

C4-related antigens and/or C1q (markers of classical pathway activation) were observed in only one of 21 and two of 22 cases of IgA-GN in whom these staining could be performed (whereas in our experience these deposits are found in 40 to 60, and 80 to 90%, respectively, of patients with lupus nephritis).

Activation of the alternative pathway was suggested in 22 of 23 cases by mesangial deposition of factor P. In eight of 22 cases, in addition, factor H was present in the deposits. Factor B was observed in one of 21 cases only. Furthermore, C3b/iC3b (C3c-related antigen) was observed in all cases of IgA-GN and with marked or striking intensity in 20 of 23 specimens. Glomeruli of all patients stained intensely with anti-C3d in a granular pattern. A mesangio-peripheral pattern of deposition was observed in 16 of 23 cases. Elution experiments performed with six biopsies, using either 1 M propionic acid or 3 M thiocyanate (for 30 min at room temperature and post-treatment with inactivated fetal calf serum to suppress non-specific binding) revealed virtually no loss of C3d. This finding suggests covalent binding of C3d.

Activation of the complete complement cascade with deposition of MAC in the mesangium (as revealed by anti-MAC neoantigens; representative examples given in Fig. 2–4) was noted in 18 of 18 cases with sufficient number of glomeruli. C5, C6 and C9 reactivity of the deposits with mesangial or mesangio-peripheral pattern was found in 19 of 19, 19 of 21 and 18 of 19 cases, respectively. Staining with anti-MAC-neo revealed the highest number of marked (2+) and striking (3+) granular deposits (16 of 18), followed by anti-C5 (16 of 19), anti-C9 (13 of 19) and anti-C6 (15 of 21). In seven cases, experiments were performed to examine whether MAC-neo and S-protein were co-localized at corresponding sites by staining with anti-MAC-neo and anti-S-protein in sequential sections. In all seven cases, the deposits virtually always stained with both anti-MAC-neo and anti-S-protein.

In four representative cases, individual sections were simultaneously examined for glomerular IgA and MAC-neo-deposits, using three different staining techniques as described in materials and methods. Figure 4 shows that the pattern of IgA staining is superimposable upon that of MAC-neo staining and that of C5b neoantigens staining (using monoclonal antibodies; data not shown). This observation suggests co-localization of immunoglobulin and complement deposits. Similar co–localization was observed for MAC and C3d.

Immunoglobulin and complement deposits in HSP

The two specimens from patients with HSP revealed quite similar immunohistological features as observed in IgA-GN (Table 2).

Deposits of antigens of the coagulation system

Fibrin deposits with mesangial or mesangio-peripheral distribution were noted in 15 of 23 cases of IgA-GN. Additionally, in four of 23 IgA-GN cases a peripheral pattern was noted. All 8 of 8 children examined, but only 11 of 15 adults with IgA-GN examined had fibrin deposits in the glomeruli. The two HSP biopsy specimens of children showed extensive mesangio-peripheral fibrin deposits. Staining of kidney biopsy specimens with FITC-labeled antibodies against fibronectin revealed a purely mesangial pattern in 15 of 29 biopsy specimens and a mesangio-peripheral pattern (Fig. 5) In 9 of 29 biopsies. Staining with anti-fibronectin of four kidney biopsy specimens with no immunohistologically–detectable immunoglobulin or complement deposits (used as controls) revealed purely mesangial deposition of fibronectin without capillary involvement.

Table 2. IF-findings in the glomeruli[a]

Case no.	IgA	IgG	IgM	Fibrin	Fibronectin	C1q	C4	C3b
IgA-glomerulonephritis								
1	3+/m	2+/mp	1+/m	3+/m	3+/m	0	0	1+/m
2	3+/m	0	1+/mp	0	3+/m	0	0	2+/m
3	3+/mp	1+/mp	1+/mp	3+/mp	3+/m	0	0	2+/m
4	3+/m	2+/m	0	3+/m	ND[c]	0	0	2+/m
5	3+/mp	1+/p	0	2+/mp	3+/mp	0	0	3+/mp
6	3+/m	1+/m	1+/mp	2+/mp	3+/m	1+/m	0	2+/m
7	3+/mp	2+/mp	1+/mp	3+/mp	3+/mp	0	0	2+/mp
8	3+/m	ND[c]	2+/m	1+/p	3+/mp	0	0	3+/m
9	3+/m	0	1+/m	0	3+/m	0	0	3+/m
10	3+/mp	2+/mp	1+/mp	3+/mp	ND[c]	0	ND[c]	3+/m
11	3+/m	2+/m	1+/m	1+/p	3+/m	2+/m	1+/m	3+/m
12	3+/m	1+/p	0	3+/m	ND[c]	0	0	1+/m
13	3+/mp	1+/p[b]	0	2+/p	ND[c]	0	0	2+/m
14	3+/mp	2+/p	1+/mp	0	3+/mp	0	0	2+/m
15	3+/m	2+/p	0	2+/m	2+/m	0	0	2+/m
16	3+/m	1+/p[b]	0	0	ND[c]	ND[c]	ND[c]	3+/m
17	3+/mp	1+/p[b]	1+/p	3+/mp	3+/mp	0	0	3+/mp
18	3+/mp	1+/p[b]	1+/mp	3+/mp	3+/mp	0	0	2+/mp
19	3+/m	1+/p[b]	0	2+/mp	3+/mp	0	0	2+/m
20	3+/m	2+/m	1+/m	2+/p	3+/m	0	0	3+/m
21	3+/m	1+/p[b]	0	3+/m	2+/m	0	0	1+/m
22	3+/mp	2+/p[b]	0	3+/m	3+/m	0	0	2+/m
23	3+/m	0	1+/m	3+/m	3+/m	0	0	2+/m
Henoch–Schönlein purpura nephritis								
24	3+/mp	2+/p[b]	1+/mp	3+/mp	3+/mp	0	0	3+/mp
25	3+/mp	2+/p[b]	1+/mp	3+/mp	3+/mp	0	0	1+/mp
Controls								
26	0	0	0	0	1+/m	0	0	0
27	0	0	0	0	2+/m	0	0	0
28	0	0	0	0	2+/m	0	0	0
29	0	0	0	0	2+/m	0	0	0

[a] Intensity of IF-staining: 0, no deposits detectable by IF; 1+, faint or weak granular deposits; 2+, marked granular deposits; 3+, extensive granular deposits. Pattern of glomerular deposition: m, exclusively mesangial pattern of granular deposits; mp, mainly mesangial granular deposits with involvement of capillary loops; p, exclusively peripheral granular deposits

[b] Pseudo-linear appearance of confluent granular peripheral deposits.

[c] No glomeruli in the cryostat section

[d] Sum of individual scores (arbitrary units) for C5 + C6 + C9 + MAC neoantigen irrespective of pattern

Statistical evaluations

In order to increase reproducibility of scoring of fluorescence patterns and intensities, the following techniques were applied: code number identification of specimens and examination without knowing the clinical diagnosis (blinding); examination of all specimens stained with one antibody under identical conditions at one time; independent examination and scoring by two experienced investigators; and photomicrography using standardized techniques.

The chi-square test was used to examine whether associations existed between the estimated pattern (peripheral vs. mesangio-peripheral) of immunoglobulin (IgA, IgG, IgM), fibronectin or C3b/iC3b deposits on the one hand and MAC-related antigens or other C-dependent antigens on the other hand.

The deposition pattern (m vs. mp) of IgA corresponded to that of C3b/iC3b ($P < 0.002$). Similarly, the IgA pattern corresponded to that of the MAC neoantigen ($P < 0.01$), of properdin ($P < 0.002$) and that of C3d($P < 0.02$); correspondence of C3b/iC3b or properdin and MAC pattern was less pronounced ($P < 0.2$ and $P < 0.05$, respectively). Correspondence of C3d and MAC pattern was closer ($P < 0.01$). (Calculations were based on all cases in whom the respective staining could be performed including both IgA-GN and HSP cases).

Deposition patterns of IgG or IgM did not correspond to that of any of the complement derived antigens.

The patterns of deposition of MAC-related antigens, that is, C5, C6, C9 and MAC neoantigens, were uniform (either all mesangial or all mesangio-peripheral) in 13 of 14 cases of IgA-GN and HSP. The sum of the scores estimated for MAC-related antigens, as defined above, correlated with histopathology: IgA-GN specimens with MGL had lower ($P = 0.05$) scores for MAC-related antigens than specimens with more advanced non-sclerosing lesions (MGN, EEGN).

No significant correlation was detected between the sum of the scores for MAC related antigens and serum creatinine or hypertension. Proteinuria correlated with the sum of MAC antigen scores ($P < 0.01$ for no proteinuria versus any degree of proteinuria).

Discussion

Although in the past, many authors (2–5, 22–33) reported deposition of C3 and some other complement (C) components

Table 2. *Continued*

Case no.	C3d	B	H	P	C5	C6	C9	MAC-neo	C5 + C6 + C9 + MAC-neo score[d]
IgA-glomerulonephritis									
1	3+/m	0	0	3+/m	3+/m	0	2+/m	2+/m	7
2	2+/mp	0	1+/m	2+/m	2+/m	0	1+/m	2+/m	5
3	3+/mp	0	1+/mp	1+/m	1+/mp	1+/mp	0	1+/mp	3
4	3+/mp	0	1+/m	1+/m	ND[c]	2+/m	ND[c]	ND[c]	
5	3+/mp	0	0	2+/mp	2+/mp	2+/mp	2+/mp	2+/mp	8
6	3+/m	2+/p	1+/m	2+/m	2+/m	2+/m	2+/m	2+/m	8
7	2+/mp	0	0	1+/m	ND[c]	ND[c]	ND[c]	ND[c]	
8	2+/mp	0	0	2+/m	2+/mp	2+/mp	2+/mp	2+/mp	8
9	3+/mp	0	0	2+/m	3+/mp	2+/mp	2+/mp	2+/mp	9
10	3+/mp	ND[c]	0	1+/m	ND[c]	2+/mp	ND[c]	ND[c]	
11	3+/mp	0	2+/m	2+/m	3+/mp	2+/mp	2+/mp	3+/mp	10
12	2+/mp	0	0	1+/m	1+/m	ND[c]	1+/m	1+/m	
13	3+/mp	ND[c]	1+/m	1+/m	2+/mp	1+/mp	ND[c]	2+/mp	
14	3+/mp	0	1+/m	2+/mp	2+/m	2+/mp	2+/mp	2+/mp	8
15	1+/m	0	0	1+/m	1+/m	2+/m	2+/m	ND[c]	
16	2+/m	0	0	2+/m	ND[c]	2+/m	2+/m	ND[c]	
17	3+/mp	0	0	2+/mp	2+/mp	2+/mp	1+/mp	2+/mp	7
18	3+/mp	0	0	2+/mp	2+/mp	2+/mp	1+/mp	2+/mp	7
19	2+/m	0	0	2+/m	3+/m	1+/m	2+/m	3+/m	9
20	3+/mp	0	0	2+/m	2+/m	2+/m	2+/m	2+/m	8
21	3+/m	0	0	1+/m	2+/m	2+/m	1+/m	2+/m	7
22	3+/mp	0	1+/mp	1+/mp	3+/mp	2+/mp	3+/mp	3+/mp	11
23	3+/m	0	0	0	3+/m	1+/m	2+/m	2+/m	8
Henoch–Schönlein purpura nephritis									
24	3+/mp	0	0	1+/mp	1+/mp	2+/mp	2+/mp	2+/mp	7
25	2+/mp	0	0	1+/mp	1+/mp	0	0	1+/mp	2
Controls									
26	ND[c]	0	0	0	0	0	0	0	0
27	2+/p	0	0	0	0	0	0	0	0
28	2+/p	0	0	0	0	0	0	0	0
29	3+/p	0	0	0	0	0	0	0	0

in the glomeruli of patients with IgA-GN or HSP, involvement of the membrane attack complex (MAC) has not been demonstrated to date in a larger series of patients with IgA-GN. However, poly-9-neoantigen was noted in the glomeruli of some (N = 3) patients IgA-GN and (N = 2) HSP in one study [34]. The present investigation gives immunohistological evidence for consistent deposition of the terminal components of the complement system (C5-9) in the mesangium and glomerular capillaries of patients with IgA-GN and HSP. Evidence for formation of such MAC is provided by the demonstration of unique neoantigens. The neoantigen(s) is (are) not present on the individual native components; they appear on several components of C5-9 when the individual components undergo complex formation and assemble to form the membrane attack complex [13]. Neoantigens are incontroversial evidence of formation of membrane attack complex. Deposition of MAC-related antigens has previously been described for other forms of human glomerulonephritis, such as membranous GN [35], anti-basement membrane GN or Goodpasture's syndrome [35] and SLE nephritis [36]. Direct [37] or indirect evidence for functional involvement of the terminal complement components has also been shown in experimental models of membranous glomerulonephritis [38–42].

Several methodological problems must be considered before our interpretation can be accepted that true neoantigens are present and that this implies generation of—at least transiently—active MAC activity. Falk et al [34] detected poly-9-neoantigen, one component of MAC, not only in immunologically-mediated glomerular lesions, but also (apparently as a result of antibody-independent complement deposition) in scar tissue and non-immune renal lesions. In agreement with this observation we detected granular and pseudo-linear (comma-like) MAC deposits on the tubular basement membranes in 16 of 21 cases. Granular MAC deposits could furthermore be detected in 16 of 21 cases intramurally in arterioles without consistent IgA deposits in either site. However, co-localization of extraglomerular MAC with C3d, that is, presence of both early and terminal components, argues against an unspecific deposition of preformed MAC at these sites.

Various tests for specificity, including immunoblotting (dot-blot analysis with purified C5b-9: present study) and immunoferritin staining [16] clearly demonstrated the high speci-

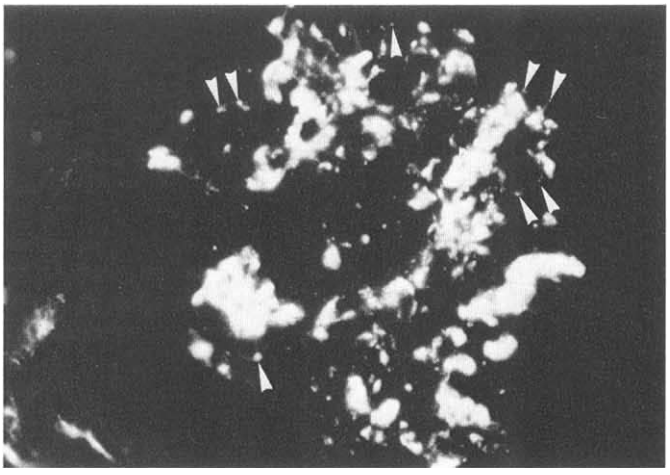

Fig. 1. *Extensive mesangial granular IgA deposits in IgA-GN (IF).* Note additional granular staining ("mp-pattern") along the peripheral capillary walls (arrowheads).

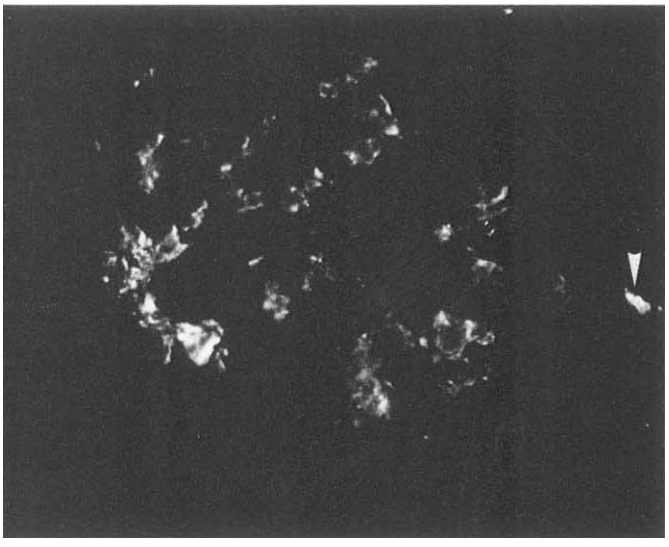

Fig. 2. *Mesangial deposits ("m"-pattern) of MAC as demonstrated by polyclonal anti-MAC neoantigen antibodies (IF).* Note the low background staining of Bowman capsule and of extraglomerular sites. The arrowhead points to a MAC deposition in a peritubular capillary wall.

ficity of our antibody for MAC neoantigens and exclude cross-reactivity with native complement components or other serum proteins. The assumption that the majority of extracellular MAC is present as SC5b-9 complex could be confirmed in selected cases immunohistologically by staining with protein S antibodies. Thus, immunohistological demonstration of MAC neoantigens in the extracellular mesangial matrix does not necessarily indicate presence of hemolytically active MAC; however, extracellular presence of MAC implies with high probability that adjacent cell membranes have been exposed to hits of functionally active MAC at concentrations below the detection threshold of the IF procedure.

Furthermore, demonstration of MAC neoantigens does not necessarily indicate antibody–dependent complement activation; the possibility must also be considered that exposure to protease activity in a glomerulonephritic mesangium might lead to antibody–independent C-activation. This possibility is considered unlikely in the present study because MAC neoantigen was uniquely co-deposited with IgA (and C3d) in the mesangium and in the glomerular capillaries. The absence of MAC in control glomeruli excludes non-specific post-biopsy in situ complement activation.

The biological consequences of MAC deposition in the mesangium are unknown. Using erythrocytes as target cells, MAC has been well characterized as a cytolytic complex which lyses cells by forming unique transmembrane channels which lead to osmotic imbalance and lysis of target cells [43]. However, nucleated cells are markedly resistent to the lytic action of MAC [44, 45]. Consequently, mechanisms other than cytolysis must be considered in the genesis of MAC-related tissue injury. Additional or alternative possibilities include an indirect modulator role of MAC in the inflammatory process, such as a signal [46] to release prostanoids (as recently demonstrated for mesangial cells [47]) or cytokine(s). It has also recently been demonstrated that MAC is internalized by nucleated cells [48]; intracellular presence of MAC opens a whole spectrum of potential intracellular actions.

Variable mesangial MAC formation may explain the paradoxical observation [49] that there is little relation between the

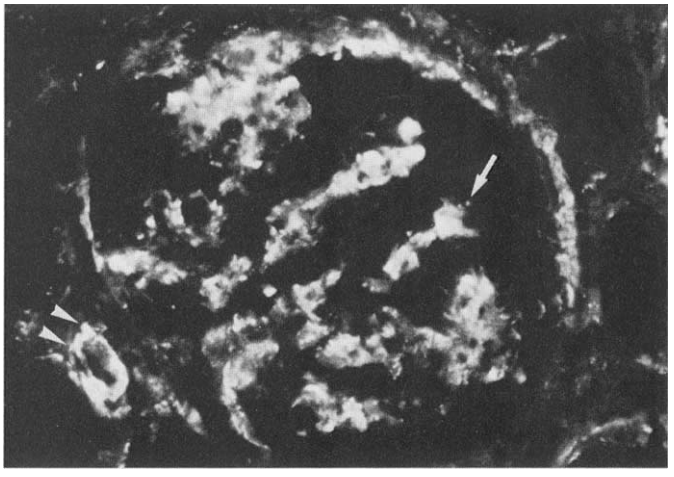

Fig. 3. *Mesangio-peripheral pattern ("mp") of MAC neoantigens.* The arrow points to granular peripheral deposits in the capillary wall. Parts of Bowmans capsule and (to a lesser degree) tubular basement membrane show additional MAC deposits. Arrowheads indicate extensive MAC deposition in a vessel wall.

amount of IgA deposited in the mesangium (as evaluated by immunofluorescence) and evidence of mesangial cell injury, for example phagocytosis, proliferation or mesangial matrix overproduction. Such discrepancy may be due to variable activation of MAC by IgA deposits or variations of elimination half life. In our study, despite presence of extensive IgA deposits on light microscopy, patients who had only mild lesions by light microscopy had less MAC deposition than patients with more advanced non-sclerosing lesions.

MAC-induced glomerular injury may not only concern mesangial cells, but also capillary walls. It is of note that involvement of glomerular capillaries by light microscopy or immunofluorescence has been shown to be associated with

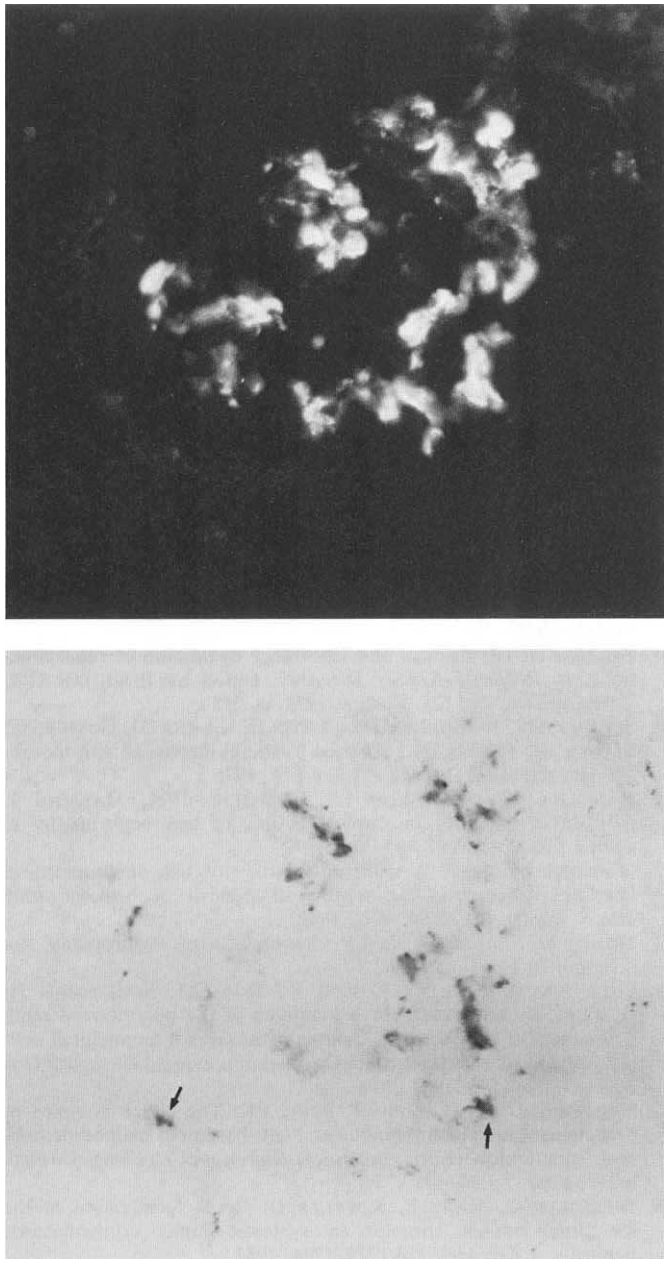

Fig. 4. *Upper part: IgA deposits in IgA-GN (IF); lower part: MAC neoantigen pattern visualized in the same section as IgA using AP-labeled secondary antibodies and substrate reaction.* Note co-localization of the majority of deposits (except IgA-independent MAC deposition in Bowmans capsule membrane (arrows)).

more severe disease and more adverse prognosis of IgA-GN [49, 50].

Experimental models of membranous GN [37–42] suggest that a relation exists between deposition of MAC in glomerular capillaries and appearance of proteinuria, either by irritation of cells or by reducing the anionic sites in the glomerular basement membrane [42]. Co-deposition of MAC and IgA in glomerular capillary walls, as indicated by the present study, would be compatible with the notion that MAC plays also a role in the

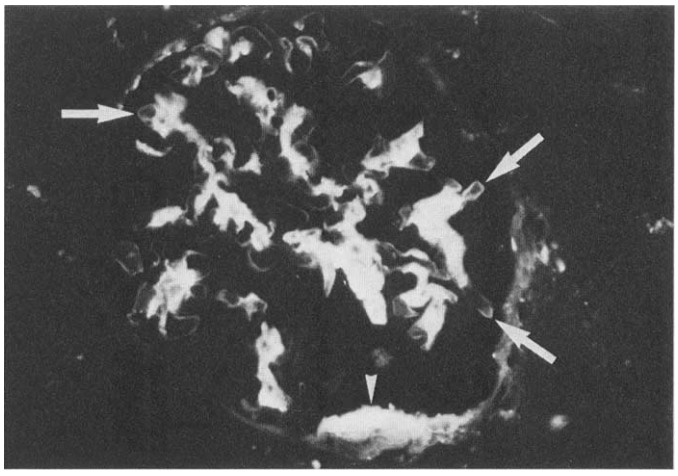

Fig. 5. *Heavy mesangial fibronectin staining with partial pseudolinear extensions into the peripheral capillaries (arrows) in IgA-GN.*

genesis of the prognostically more adverse capillary wall lesions.

The pattern of MAC deposition, that is mesangial or mesangio-peripheral, corresponded to that of IgA deposition, but not of IgG and IgM deposition (the latter of which was always less intense). This suggests that IgA aggregates or IgA immunocomplexes are involved in the terminal activation of complement components.

In the past, there has been much controversy whether IgA is capable to activate the C-system. Activation of the alternative pathway of complement in vitro by aggregated IgA and IgA-IC has been found by some [51–56] but not all authors [57–59]. More recent observations, however, confirm C-activation by IgA-IC: mouse myeloma IgA, forming immune complexes with DNP-BSA, was shown to activate complement and to induce local C-dependent lung injury in an Arthus-like rat model [60]. While evidence for C-activation by IgA-IC is incontroversial in this model, we would like to caution, with respect to mesangial IgA, that a role for the (unknown) antigen(s) or immunoglobulins of other Ig-classes in the genesis of complement activation has not been finally excluded.

With respect to the pathway involved, most authors [25, 33, 61] agree that—as also noted in this study—C3b and factor P are frequently co-deposited with IgA in the absence of noticeable C1q and C4. This suggests that in IgA-GN and HSP C-activation occurs mainly or exclusively via the alternative pathway. Deposits of C3d, and less consistently of C3b/iC3b, corresponded topographically to those of MAC-neo. Resistance of C3d against drastic conditions of elution suggests covalent binding of C3d and strongly argues against non-specific binding. The correspondence of C3d and MAC-neo patterns, such as patterns of early and terminal C-components, is a strong argument for complete local C-activation.

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Reprint requests to Professor Dr. E. W. Rauterberg, Institut für

Immunologie und Serologie, Immunpathologie, Im Neuenheimer Feld 305, 6900 Heidelberg 1, Federal Republic of Germany.

References

1. Berger J, Hinglais N: Les depôts intercapillaries d'IgA-IgG. *J Urol Nephrol* 74:694–695, 1968
2. Lowance DC, Mullins JD, McPhaul JJ: Immunoglobulin A (IgA) associated glomerulonephritis. *Kidney Int* 3:167–176, 1973
3. Berger J: Idiopathic mesangial depositions of IgA, in *Nephrology* edited by *Hamburger J, Crosnier J, Grunfeld JP*. New York, John Wiley & Sons, 1979, p. 535
4. Woodroffe AJ, Clarkson AR, Seymour AE, Lomax-Smith JD: Mesangial IgA nephritis. *Springer Semin Immunopathol* 5:321–332, 1982
5. Heaton JM, Turner DR, Cameron JS: Localization of glomerular 'deposits' in Henoch–Schönlein nephritis. *Histopathology* 1:93–104, 1977
6. Levinsky RJ, Barratt TM: IgA immune complexes in Henoch–Schönlein purpura. *Lancet* ii:1100–1103, 1979
7. Hall RP, Lawley TJ, Heck JA, Katz SI: IgA-containing circulating immune complexes in dermatitis herpetiformis, Henoch–Schönlein purpura, systemic lupus erythematosus and other diseases. *Clin Exp Immunol* 40:431–437, 1980
8. Kauffmann RH, Herrmann WA, Meyer CJLM, Daha MR, Van Es LA: Circulating IgA-immune complexes in Henoch–Schönlein purpura. A longitudinal study of their relationship to disease activity and vascular deposition of IgA. *Am J Med* 69:859–866, 1980
9. Woodroffe AJ, Gormly AA, McKenzie PE, Wootton AM, Thompson AJ, Seymour AE, Clarkson AR: Immunologic studies in IgA nephropathy. *Kidney Int* 18:366–374, 1980
10. Lesavre P, Digeon M, Bach JF: Analysis of circulating IgA and detection of immune complexes in primary IgA nephropathy. *Clin Exp Immunol* 48:61–69, 1982
11. Hall RP, Stachura I, Cason J, Witheside TL, Lawley TJ: IgA-containing circulating immune complexes in patients with IgA-nephropathy. *Am J Med* 74:56–63, 1983
12. Valentijn RM, Kauffmann RH, De La Rivière GB, Daha MR, Van Es LA: Presence of circulating macromolecular IgA in patients with hematuria due to primary IgA nephropathy. *Am J Med* 74:375–381, 1983
13. Kolb WP, Müller-Eberhard HJ: Neoantigens of the membrane attack complex of human complement. *Proc Natl Acad Sci USA* 72:1687–1689, 1975
14. Rautherberg EW, Hänsch GM, Rother U: Isolation of late complement components by affinity chromatography. II. Purification of the human complement component C6. *Immunobiol* 156:142–152, 1979
15. Ross GD, Lambris JD, Cain JA, Newman SL: Generation of three different fragments of bound C3 with purified factor I or serum. I. Requirements for factor H vs CR_1 cofactor activity. *J Immunol* 129:2051–2060, 1982
16. Hegemann P: Assoziation der Komplementfaktoren C5, C6, C8 und der C5-9 Neoantigene an Ringstrukturen auf komplementlysierten Membranen: Eine elektronenoptische, morphometrische Untersuchung mit Hilfe der Immunoferritin Methode. Thesis, University of Heidelberg, 1982
17. Balcarova-Ständer J, Rother U, Rauterberg EW: The attack phase of human complement: Differentiation between membrane binding and complex formation by the detection of neoantigen expression in situ. A morphometric immunoferritin study. *J Immunol* 127:1089–1093, 1981
18. Levy LR, Sober HA: A simple chromatographic method for the preparation of gamma globulin. *Proc Soc Exp Biol Med* 103: 250–252, 1960
19. McCluskey RT, Vassalli P, Gallo G, Baldwin DS: An immunofluorescent study of pathogenic mechanisms in glomerular diseases. *N Engl J Med* 274:695–701, 1966
20. Moongkarndi P: Monoclonal antibodies against the fifth component of human complement. Thesis, University of Heidelberg, 1984
21. Cordell JL, Falini B, Erber WN, Ghosh AK, Abdulaziz Z, MacDonald S, Pulford KAF, Stein H, Mason DY: Immunoenzymatic labeling of monoclonal antibodies using immune complexes of alkaline phosphatase and monoclonal anti–alkaline phosphatase (APAAP complexes). *J Histochem Cytochem* 32: 219–229, 1984
22. Larsen S: Immunofluorescent microscopy findings in minimal or no change–disease and slight generalised mesangioproliferative glomerulonephritis. *Acta Path Microbiol Scand* Sect. A 86: 531–542, 1978
23. Shigematsu H, Kobayashi Y, Tateno S, Hiki Y, Kuwao S: Glomerular tissue injury in IgA nephritis. *Acta Pathol Jpn* 33: 367–380, 1983
24. Nagy J, Brasch H, Süle T, Hámori A, Deák G, Ambrus M: IgA glomerulonephritis: Mesangial IgA deposition without systemic signs. *Int Urol Nephrol* 11:367–375, 1979
25. Tomino Y, Sakai H, Miura M, Endoh M, Nomoto Y: Detection of polymeric IgA in glomeruli from patients with IgA nephropathy. *Clin Exp Immunol* 49:419–425, 1982
26. Tomino Y, Nomoto Y, Endoh M, Sakai H, Arimori S: Double immunofluorescence studies on IgA-associated immune–complexes in glomerular deposits in patients with IgA nephropathy. *Tokai J Exp Clin Med* 5:147–149, 1980
27. Moriconi L, Paleologo G, Bedini L, Cioni L: IgA nephropathy. Clinical, pathological and immunological findings. *La Ricerca Clin Lab* 9:369–377, 1979
28. Shirai T, Tomino Y, Sato M, Yoshiki T, Itoh T: IgA nephropathy: Clinicopathology and immunopathology. *Contr Nephrol* 9: 88–100, 1978
29. Goldsmith DI: Clinical and laboratory evaluation of renal functions, in *Pediatric Kidney Diseases*, edited by Edelman CM. Little, Brown, and Co. Boston, 1978, p. 213
30. Sissons JGP, Woodrow DF, Curtis JF, Evans DJ, Gower PE, Sloper JC, Peters DK: Isolated glomerulonephritis with mesangial IgA deposits. *Br Med J* 3:611–614, 1975
31. Van Der Peet J, Arisz L, Brentjens JRH, Marrink J, Hoedemaeker PJ: The clinical course of IgA nephropathy in adults. *Clin Nephrol* 8:335–340, 1977
32. Hogg RJ, Silva F: A multicenter study of IgA nephropathy in children. A report of the southwest pediatric nephrology study group. *Kidney Int* 22:643–652, 1982
33. McCoy RC, Abramowsky CR, Tisher CC: IgA nephropathy. *Am J Pathol* 76:123–140, 1974
34. Falk RJ, Dalmasso AP, Kim Y, Tsai CH, Scheinman JI, Gewurz H, Michael AF: Neoantigen of the polymerized ninth component of complement. Characterization of a monoclonal antibody and immunohistochemical localization in renal disease. *J Clin Invest* 72:560–573, 1983
35. Rauterberg EW, Gehrig T, Kohl PK: The attack complex of complement in epimembranous and anti-basement membrane antibody glomerulonephritis. (abstract) *Nieren und Hochdruckkrankh* 9:217, 1980
36. Biesecker G, Katz S, Koffler D: Renal localization of the membrane attack complex in systemic lupus erythematosus nephritis. *J Exp Med* 154:1779–1794, 1981
37. Groggel GC, Adler S, Rennke HG, Couser WG, Salant DJ: Role of the terminal complement pathway in experimental membranous nephropathy in the rabbit. *J Clin Invest* 72:1948–1957, 1983
38. Adler S, Baker PJ, Pritzl P, Couser WG: Detection of terminal complement components in experimental immune glomerular injury. *Kidney Int* 26:830–837, 1984
39. De Heer E, Daha MR, Bhakdi S, Bazin H, Van Es LA: Possible involvement of terminal complement complex in active Heymann nephritis. *Kidney Int* 27:388–393, 1985
40. Koffler D, Biesecker G, Noble B, Andres GA, Martinez-Hernandez A: Localization of the membrane attack complex (MAC) in experimental immune complex glomerulonephritis. *J Exp Med* 157:1885–1905, 1983
41. Biesecker G, Noble B, Andres GA, Koffler D: Immunopathogenesis of Heymann's nephritis. *Clin Immunol Immunopathol* 33:333–338, 1984
42. Parra G, Takekoshi Y, Vernier RL: Association of localization of poly C9 with proteinuria and local loss of anionic sites in the glomerular basement membrane (GBM) in acute serum sickness nephritis. (abstract) *Am Soc Nephrol* 121A, 1984

43. MAYER MM: Mechanism of cytolysis by complement. *Proc Natl Acad Sci USA* 69:2954–2958, 1972
44. SCHLAGER SI, OHANIAN SH, BORSOS T: Correlation between the ability of tumor cells to resist humoral immune attack and their ability to synthesize lipid. *J Immunol* 120:463–471, 1978
45. SCHLAGER SI, OHANIAN SH, BORSOS T: Identification of lipids associated with the ability of tumor cells to resist humoral immune attack. *J Immunol* 120:472–480, 1978
46. HÄNSCH GM, SEITZ M, MARTINOTTI G, BETZ M, RAUTERBERG EW, GEMSA D: Macrophages release arachidonic acid, prostaglandin E2, and thromboxane in response to late complement components. *J Immunol* 133:2145–2150, 1984
47. LOVETT D, HÄNSCH GM, RESCH K, GEMSA D: Activation of glomerular mesangial cells (MC) by terminal complement components: Stimulation of prostanoid and interleukin 1 (IL-1)-like factor release. *Immunobiol* 168:34–35, 1984
48. CARNEY DF, KOSKI CL, SHIN ML: Elimination of terminal complement intermediates from the plasma membrane of nucleated cells: The rate of disappearance differs for cells carrying C5b-7 or C5b-8 or a mixture of C5b-8 with a limited number of C5b-9. *J Immunol* 134:1804–1809, 1985
49. D'AMICO G, FERRARIO F, COLASANTI G, RAGNI A, BESTETTI BOSISIO M: IgA-mesangial nephropathy (Berger's disease) with rapid decline in renal function. *Clin Nephrol* 16:251–257, 1981
50. LARSEN S: Immune deposits in generalised mesangioproliferative glomerulonephritis. *Acta Path Microbiol Scand* Sect. A 86: 543–552, 1978
51. GÖTZE O, MÜLLER-EBERHARD HJ: The C3-activator system: An alternate pathway of complement activation. *J Exp Med* 134: 90s–108s, 1971
52. PFAFFENBACH G, LAMM ME, GIGLI I: Activation of the guinea pig alternative complement pathway by mouse IgA immune complexes. *J Exp Med* 155:231–247, 1982
53. SPIEGELBERG HL, GÖTZE O: Conversion of C3 proactivator and activation of the alternate pathway of complement activation by different classes and subclasses of human immunoglobulins. (abstract) *Fed Proc* 31:655, 1972
54. FRANK MM, GAITHER T, ADKINSON F, TERRY WD, MAY JE: Activation of the alternate complement pathway by human immunoglobulins. (abstract) *J Immunol* 116:1733 (A), 1976
55. MILLER GW: Solubilization of IgA immune precipitates by complement. *J Immunol* 117:1374–1377, 1976
56. TOMINO Y, ENDOH M, NOMOTO Y, SAKAI H: Activation of complement by renal tissues from patients with IgA nephropathy. *J Clin Pathol* 34:35–40, 1981
57. ISHIZAKA K, ISHIZAKA T, LEE EH, FUDENBERG H: Immunochemical properties of human gammaA isohemagglutinin. I. Comparisons with gammaG- and gammaM-globulin antibodies. *J Immunol* 95:197–208, 1965
58. IIDA K, FUJITA T, INAI S, SASAKI M, KATO T, KOBAYASHI K: Complement fixing abilities of IgA myeloma proteins and their fragments: The activation of complement through the classical pathway. *Immunochem* 13:747–752, 1976
59. BURRITT MF, CALVANICO NJ, MEHTA S, TOMASI TB: Activation of the classical complement pathway by Fc fragment of human IgA. *J Immunol* 118:723–725, 1977
60. JOHNSON KJ, WILSON BS, TILL GO, WARD PA: Acute lung injury in rat caused by immunoglobulin A immune complexes. *J Clin Invest* 74:358–369, 1984
61. EVANS DJ, WILLIAMS DG, PETERS DK, SISSONS JGP, BOULTON-JONES JM, OGG CS, CAMERON JS, HOFFBRAND BI: Glomerular deposition of properdin in Henoch–Schönlein syndrome and idiopathic focal nephritis. *Br Med J* 3:326–328, 1973